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Screening broad beans (*Vicia faba*) for magnesium deficiency. II. Photosynthetic performance and leaf bioelectrical responses

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Abstract. In search of rapid screening tools for magnesium (Mg) deficiency in crops at early stages of plant ontogeny, we studied the kinetics of leaf photosynthetic responses and changes in electrophysiological characteristics of broad bean leaves as affected by different levels of Mg in the nutrient solution (1–200 ppm). No apparent correlation between plant age, Mg supply level, leaf stomatal conductance (g_s) and transpiration rate (E) were found. A significant difference in CO_2 assimilation became obvious only at week 8. Chlorophyll fluorescence analysis, however, revealed a significant difference in the maximal quantum efficiency of PSII (F_v/F_m ratio) between Mg-deficient and Mg-sufficient plants as early as 2 weeks after seedling emergence. The most sensitive measurements were of light-induced changes in the leaf surface electric potential, with an almost 2-fold difference in the magnitude of leaf bioelectric response between 10 ppm (deficient) and 50 ppm (optimal) treatments. Preliminary experiments in which net Mg^{2+} fluxes were measured using the non-invasive ion flux estimation (MIFE) technique showed that the electrical changes on the leaf surface might, to some extent, reflect the movement of Mg^{2+} across the plasma membrane.

Keywords: deficiency, electric potentials, ion fluxes, magnesium, photosynthesis, screening, transpiration, *Vicia faba*.

Introduction

A previous study (Hariadi and Shabala 2004) showed that neither growth kinetics nor visual deficiency symptoms were good indicators for diagnostics of Mg deficiency in broad beans. The use of leaf elemental analysis for Mg content is also too costly and time-consuming, despite its apparent sensitivity. In search for efficient and reliable ways of early diagnosis of Mg deficiency in crops, assessment of leaf photosynthetic activity might be employed.

Magnesium is central to leaf photosynthesis. In addition to being a central part of the chlorophyll molecule, Mg regulates activity of several key photosynthetic enzymes involved in carbon fixation (Marschner 1995; Mehne-Jakobs 1995). Another important function of Mg is its involvement in phloem loading and export of photosynthates from source leaves (Cakmak *et al*. 1994*a*, *b*). Magnesium availability affects the size, structure and function of chloroplasts, including electron transfer in PSII (Lavon and Goldschmidt 1999). Activity of ribulose-1,5 bisphosphate (RuBP) carboxylase in the chloroplast stroma is strongly dependent on pH and Mg. Binding Mg to this enzyme increases its affinity (K_m) for the substrate CO_2 and the turnover rate V_{max} (Marschner 1995). Magnesium also shifts the pH optimum of the reaction towards the physiological range.

Accumulation of photosynthase in leaves exerts a feedback regulation on RuBP carboxylase/oxygenase in favour of the oxygenase reaction and, thus, enhances O_2 activation (Marschner 1995). Accordingly, in Mg-deficient leaves the formation of superoxide radicals (O_2^-) and hydrogen peroxide (H_2O_2) is enhanced (Cakmak 1994). Magnesium-deficient leaves, therefore, are highly photosensitive, and symptoms of chlorosis and necrosis strongly increase with the light intensity to which the leaves are exposed.

From the above, one might expect that leaf photosynthetic responses might be severely altered when plants are grown under low Mg conditions. Accordingly, the aim of this work was to study the kinetics of development of Mg deficiency symptoms and make a comparative evaluation of several photosynthetic characteristics as prospective tools for early diagnosis of Mg deficiency in broad beans. Net CO_2 assimilation, stomatal conductance, and chlorophyll fluorescence were measured at regular intervals from plants

Abbreviations used: A, net rate of CO_2 assimilation; DW, dry weight; E, transpiration rate; ETR, electron transport rate; F_v/F_m, maximum quantum efficiency of PSII; g_s, stomatal conductance to water vapor; IRGA, infra red gas analyser; LIX, liquid ion exchanger; MIFE, microelectrode ion flux estimation; MP, membrane potential; NPQ, non-photochemical quenching; PPFD, photosynthetic photon flux density; Y, quantum yield of PSII.

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grown in a range of Mg concentrations from 1 ppm (severely deficient) to 200 ppm (excessive). Also, it is widely reported that the plasma membrane is involved in almost every aspect of cellular growth and metabolism, including adaptive responses to environmental stresses (Zimmermann *et al.* 1999). As such it would be expected that leaf Mg status be reflected in altered activity of plasma membrane transporters. To test this hypothesis, we measured light-induced changes in leaf bioelectric potentials as an overall assessment of Mg effects on the activity of plasma membrane transporters during dark/light transitions.

Materials and methods

Plant material and growth conditions

Broad beans (*Vicia faba* L. cv. Coles Dwarf, Cresswell's Seeds, New Norfolk, Australia) were grown hydroponically from seeds in a 70:30% (v/v) sterilised sand:perlite mix in a glasshouse as described in our previous publication (Hariadi and Shabala 2004). Plants were grown at six different magnesium concentrations [1, 10, 20, 50 (control), 80 and 200 ppm] as described previously (Hariadi and Shabala 2004).

Photosynthesis, transpiration and leaf conductance

Net rate of CO_2 assimilation (A), stomatal conductance to water vapour diffusion (g_s) and leaf transpiration rate (E) were measured on a monthly basis with a portable infrared gas analyser, IRGA (LCi-003 Analyser, ADC BioScientific Ltd, Hertfordshire, UK). All measurements were made on the fifth fully expanded leaf in a glasshouse using an artificial light source (400 W MF400 L/BU halogen lamp, GEC Pty Ltd, Sydney, NSW). During measurements, the leaf temperature in the cuvette was 26 ± 3°C, and the reference CO_2 concentration at approximately 370 mg L^{-1}. Leaf photosynthetic characteristics were first measured at full irradiance (between 1600 and 1800 μmol m^{-2} s^{-1}), then photosynthetic photon flux density (PPFD) was reduced by approximately 30% by putting several layers of shade cloth (nylon mesh of 1 mm^2 hole size) over the cuvette, and measurements were repeated. In total, for each individual leaf, all photosynthetic parameters (A, g_s, and E values) were obtained for five or six levels of PPFD.

Chlorophyll fluorescence

Chlorophyll *a* fluorescence parameters were measured *in vivo* from the 5th bean leaf at a temperature of 24 ± 2°C using a pulse-amplitude modulation portable fluorometer (Mini-PAM, Heinz Walz GmbH, Effeltrich, Germany). All measurements were made in the saturation pulse method described in the Mini-PAM manual. Plants were dark-adapted for 15–20 min. The F_0 value was then measured under low irradiance (0.15 μmol quanta m^{-2} s^{-1}) modulated measuring beam and F_m (maximal fluorescence) was induced by a 800-ms pulse of intense saturating white light (8000 μmol m^{-2} s^{-1}). Variable to maximum fluorescence ratio was then calculated as $F_v/F_m = (F_m - F_0)/F_m$. When measurements were taken from light-adapted samples, the leaf was illuminated by white actinic light (180 μmol m^{-2} s^{-1}) for 30 s to reach the steady-state F_0' value, and then saturated pulse was applied. Photochemical (qP) and non-photochemical (qN) quenching coefficients were then calculated using the following equations (Shabala 2002):

$$qP = (F_m' - F_0')/(F_m' - F_0) \quad (1)$$

$$qN = (F_m - F_m')/(F_m - F_0) \quad (2)$$

Measurements were taken at regular intervals (every 2 weeks) commencing from the second week after seedling emergence. Leaves from six to eight individual plants were measured for each treatment.

Bioelectric measurements

Light-induced changes in leaf surface bioelectric potentials were measured using the standard extracellular electrodes as described by Shabala (1997). Twelve leaves (usually from four different Mg treatments; three replicates each) were measured simultaneously using the 12-channel data logger (Helec DataTaker800, Hocking Electronics, Hobart, Tas.). Leaves were excised from plants grown at different Mg concentrations (as described above) 1–2 h before measurements. Petioles of excised leaves were immediately immersed into measuring solution, and kept at ambient room illumination. For measurements, leaves were put in contact with a non-polarising Ag/AgCl electrode by means of an electrolytic bridge (Shabala 1997). The transparent perspex clip of approximately 2 mm width gently pressed a wet cotton wick to the lower epidermis of the measured plant leaf. The wick was immersed in a solution containing 0.1 mM KCl and 0.1 mM $CaCl_2$ (pH 6.0) from the top of the sensor body. In the bottom of the body was a nylon tube filled with 2% agar in 1 M KCl with an impaled chlorided silver wire. The electrode was connected to a data logger. All measurements were performed in a light-tight Faraday cage.

Light treatment was given as a light ON/OFF step-wise function, of 20 min duration. Osram Dulux EE (Chatswood, NSW) neutral white cold light globes were used, providing light intensity of 900 W m^{-2}. All experiments were conducted in a temperature controlled room at 22 ± 1°C. The reference electrode was a non-polarising Ag/AgCl glass micro-capillary, with a tip diameter of approximately 50 μm, filled with 1% agar made up in 1 M KCl. Data acquisition frequency was 1 Hz. Altogether, 8–12 replicates for each treatment were measured for four different Mg concentrations (1, 10, 50 and 200 ppm) from the mid-lamella of the fifth bean leaf at plant age of 2, 3, 4, and 8 weeks.

Mg^{2+} flux measurements

Net Mg^{2+} fluxes were measured from the mesophyll cells of broad bean leaves grown at optimal (50 ppm) Mg concentrations using non-invasive microelectrode ion flux estimation (MIFE) technique. The Mg^{2+} electrode was fabricated following the standard protocol (see Shabala *et al.* 1997 or Shabala 2000 for specific details). Backfilling solution was 0.5 M $MgCl_2$. Fluka (63048) Cocktail A was used as the Mg-selective ionophore. To avoid the problem of Mg LIX sensitivity to Ca^{2+}, the latter was omitted from the measuring solution.

To measure Mg^{2+} flux bean leaves were excised, their epidermis removed, and mesophyll segments of approximately 5 × 7 mm size cut and left floating on aerated nutrient solution (0.1 mM $CaCl_2$ + 0.2 mM KCl) as described by Shabala *et al.* (2000). Each segment was then placed in a plastic holder that provided a gentle bending of the plant tissue, and allowed a clear view for electrode positioning compared with planar leaf arrangement (Shabala and Newman 1999). The holder was installed in the measuring chamber of 10 mL volume. The chamber was filled with solution and fixed on the hydraulic micromanipulator under the microscope. The dim green microscope light of approximately 12 W m^{-2} was used as the background illumination tangential to the leaf surface. Experiments started 1 h after plants were dim-light-adapted.

Results

At the age of 4 weeks, light curves of photosynthesis were essentially the same for all treatments, with the optimal Mg50 treatment showing slightly lower A values under high irradiances (Fig. 1*A*). Further measurements suggested the latter fact can be partially explained by Mg effects on stomatal conductance, g_s (Fig. 2). At the age of 4 weeks, g_s values for Mg1 leaves were two times higher than for all other treatments, including the optimal Mg50. At the age of

8 weeks, stomatal conductance of Mg-deficient leaves had not changed significantly, while in plants grown at optimal Mg supply (Mg50) g_s values were significantly higher, especially at higher PPFD (Fig. 2*B*). At this age, severe Mg deficiency (Mg1) resulted in several-fold decrease in net CO_2 assimilation. However, at the same time, it was not possible to distinguish between moderately-deficient (Mg10) and optimal (Mg50) treatments (Fig. 1*B*). Only 12 weeks after commencing the experiment, plant photosynthetic responses were 'ranked' according to Mg nutritional status (Fig. 1*C*).

Transpiration data revealed that at age 4 and 12 weeks, Mg50 plants showed double the rates of transpiration compared with Mg1 (Fig. 3*A*, *C*), while in 8-week-old plants, the trend was reversed (Fig. 3*B*). Overall, a very strong positive correlation was found to exist between net CO_2 assimilation, stomatal conductance and leaf transpiration (Table 1). At the same time, none of these characteristics were strongly correlated with leaf Mg content (Table 2). However, a strong correlation between leaf transpiration rate (*E*) and leaf K^+ content (see Hariadi and Shabala 2004) was found (Table 2).

Chlorophyll fluorescence characteristics were more responsive to plant Mg status (Table 3; Fig. 4). A significant (P=0.001) reduction in maximum photochemical efficiency of PSII (F_v/F_m values) was observed in severely Mg-deficient plants (Mg1) as early as 2 weeks after germination, in dark-adapted samples (Table 3). The effect

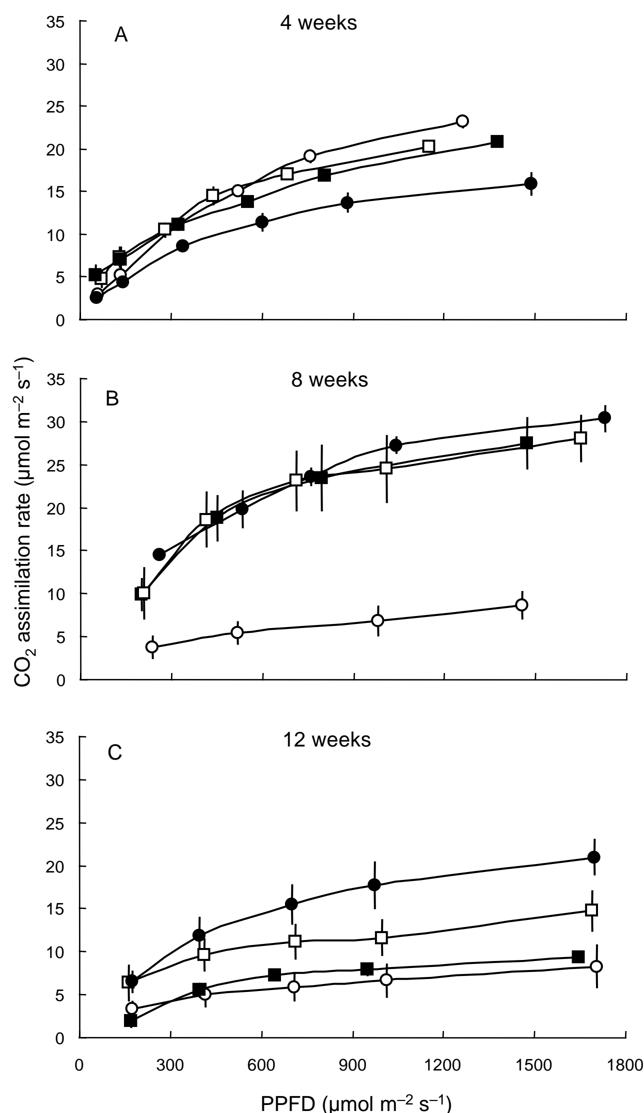

Fig. 1. Net CO_2 assimilation (μmol CO_2 m^{-2} s^{-1}) measured from the 5th broad bean leaf after 4, 8, and 12 weeks of plant growth, as a function of photosynthetic photon flux density and magnesium availability (○, Mg1; □, Mg10; ●, Mg50; ■, Mg200). Data are average ± SE (n = 5 or 6).

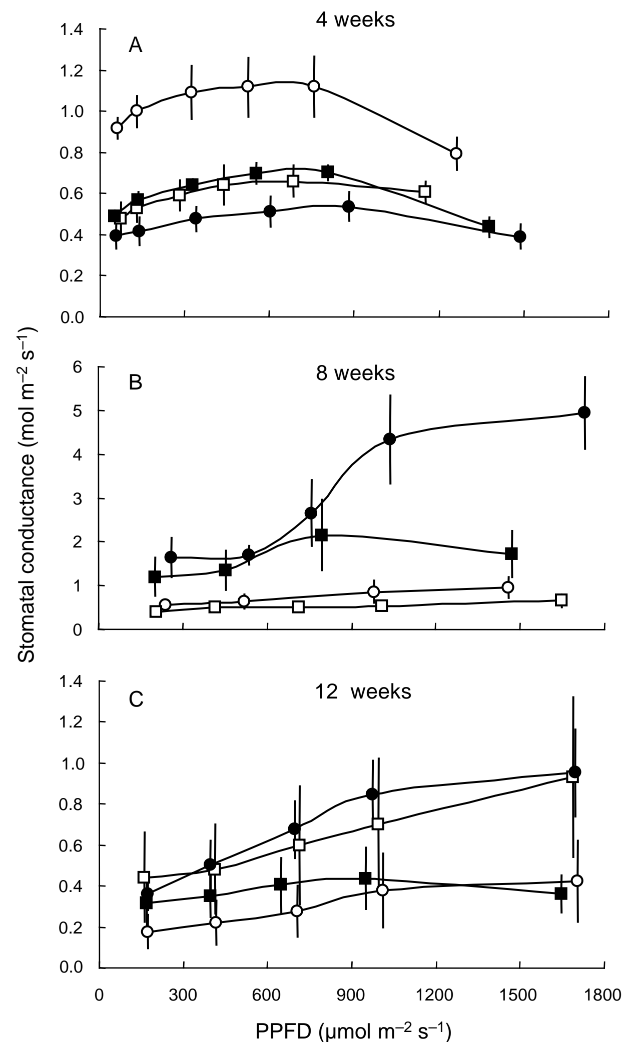

Fig. 2. Leaf stomatal conductance to water vapour (mol m^{-2} s^{-1}) measured from the 5th broad bean leaf after 4, 8, and 12 weeks of plant growth, as a function of photosynthetic photon flux density and magnesium availability (○, Mg1; □, Mg10; ●, Mg50; ■, Mg200). Data are average ± SE (n = 5 or 6).

became even more pronounced as plants grew, and a significant (P=0.001) difference between Mg1 and all other treatments was observed for F_v/F_m at any time (Table 3; Fig. 4). Measurements on light-adapted samples revealed a significant impact of Mg availability on quantum yield Y and quenching characteristics (Fig. 4). The yield and photochemical quenching (qP) were consistently lower in deficient plants, while non-photochemical quenching (qN) was higher.

In search for an efficient method of early screening for Mg deficiency, leaf surface electrical potentials were measured in response to light treatments. Onset of illumination (cold white light; 900 W m^{-2}) caused a complex multiphase transient response in all leaves (Fig. 5). It started as a rapid (approximately 30 s) depolarisation followed by more prolonged (3–5 min) hyperpolarisation, followed by a series of damped oscillations. The magnitude of response (a difference between steady-state value and a peak value at maximum hyperpolarisation) showed a very strong correlation with Mg availability for growth (Fig. 5). As early as 2 weeks after treatment, a significant difference between Mg50 and all other treatments was observed (Fig. 5*C*). The same pattern of response was measured at age 4 (Fig. 5*C*) and 8 (data not shown) weeks.

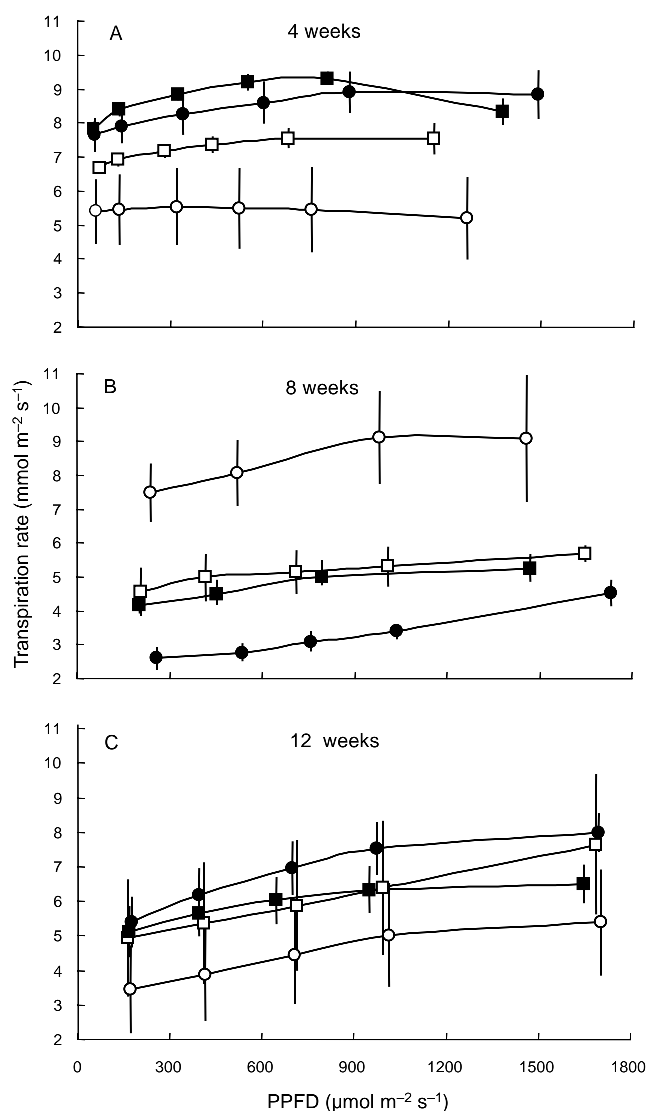

Fig. 3. Transpiration rate (mmol of H_2O m^{-2} s^{-1}) measured from the 5th broad bean leaf after 4, 8, and 12 weeks of plant growth, as a function of photosynthetic photon flux density and magnesium availability (○, Mg1; □, Mg10; ●, Mg50; ■, Mg200). Data are average ± SE (n = 5 or 6).

In order to understand physiological mechanisms underlying leaf electrical response to light, described above, net Mg^{2+} fluxes were measured near the leaf mesophyll segments of plants, grown in Mg50 solution. Onset of illumination caused rapid and significant Mg^{2+} uptake (Fig. 6*A*). A strong (> 0.8) correlation between light-induced Mg^{2+} influx and changes in leaf surface potential (Fig. 6*B*) was found for the 30 s of light treatment. It is likely, therefore, that observed changes in leaf surface potential may be at least partially attributed to light-induced Mg^{2+} transport across the plasma membrane.

Discussion

Magnesium deficiency-induced depression of photosynthesis is a widely reported phenomenon observed in a wide range of species (Bottrill *et al*. 1970; Cakmak *et al*. 1994*a*; Fischer 1997; Troyanos *et al*. 1997; Sun and Payn 1999; Ridolfi and Garrec 2000). In some of the species, such as pine, net CO_2 assimilation rates (A) and leaf conductance (g_s) were found to relate to shoot Mg concentration (Sun and Payn 1999). However, in the present study we found no strong correlation between leaf Mg content and A, g_s or E

Table 1. Correlation between changes in net CO_2 assimilation, leaf transpiration and stomatal conductance in responses to increased PPFD

Measurements were taken on the 5th broad bean leaf after 4, 8, and 12 weeks of plant growth at various levels of magnesium availability. A, net CO_2 assimilation rate; E, leaf transpiration; g_s, stomatal conductance

Pair/treatment	Mg1	Mg10	Mg50	Mg200
4 weeks				
A and E	0.50	0.99	1.00	0.97
E and g_s	0.77	0.99	0.99	1.00
A and g_s	0.93	0.99	1.00	0.95
8 weeks				
A and E	0.92	0.96	0.92	0.97
E and g_s	0.96	0.98	0.88	0.78
A and g_s	0.98	0.90	0.99	0.73
12 weeks				
A and E	0.98	0.96	1.00	0.98
E and g_s	0.99	1.00	1.00	0.72
A and g_s	0.97	0.94	0.99	0.68

Table 2. Correlation between leaf nutritional status (Mg and K content in the 5th leaf) and net CO_2 assimilation (A), leaf transpiration rate (E) and stomatal conductance (g_s)
Measurements were taken at saturating light intensities. Data for leaf Mg and K content was taken from Hariadi and Shabala (2004)

Age/pair	Mg and A	Mg and E	Mg and g_s	K and A	K and E	K and g_s
4 weeks	–0.44	0.83	–0.47	0.40	–0.79	0.40
8 weeks	0.48	–0.55	0.22	–0.72	0.80	–0.95
12 weeks	–0.06	0.11	–0.36	0.36	0.66	0.25

values (Table 2). The possible explanation may lie in the fact that most of the reported measurements were taken on plants exhibiting pronounced visual symptoms of Mg deficiency (Sun and Payn 1999), while in our study visible symptoms of Mg deficiency became evident only at a later stage of plant ontogeny (after 8 weeks; see Fig. 4 in Hariadi and Shabala 2004).

Stomatal v. *non-stomatal limitation of photosynthesis*

There are many reports implicating the overall reduction of net CO_2 assimilation to reduced stomatal conductance, g_s (Fischer and Bremer 1993; Fischer 1997; Sun *et al*. 2001). After 11 d of severe Mg deficiency, bean leaves showed severe interveinal chlorosis and spot-like necrosis, and had their stomatal aperture at almost zero values (Fischer 1997). A reduction of stomatal conductance was suggested as a major reason for the reduction in net CO_2 assimilation in response to Mg deficiency in *Pinus radiata* (Sun *et al*. 2001**)**. In the present study, we have found a very strong positive correlation (> 0.9) between A and g_s for most of the treatments throughout the experiment (Table 1).

Despite observations of coupled reduction of photosynthesis and stomatal conductance, there have been suggestions that non-stomatal limitation of photosynthesis may play a more prominent role in Mg-deficient plants. Sun and Payn (1999) suggested that reduced stomatal conductance of severely Mg-deficient pine trees should be considered as an artefact caused by needle damage. Cakmak *et al*. (1994*a, b*) showed that Mg has a distinct role in the export of photosynthates from leaves to roots. The accumulation of carbohydrates leads to reduced activities of Calvin-cycle enzymes (Krapp *et al*. 1991; Mehne-Jakobs 1995). Under

Table 3. Chlorophyll fluorescence parameters measured from the dark-adapted leaves of broad bean plants after 2, 4, 8, and 12 weeks of plant growth at various concentrations of magnesium level
Data are average ± SE (n = 8 to 12). F_v/F_m, maximum quantum yield of PSII; F_0, minimal fluorescence of dark-adapted sample; F_m, maximal fluorescence of dark-adapted sample. Levels of significance compared with control (Mg50) are indicated: *,P=0.05; **, P=0.01; ***P=0.001

Treatment	Plant age (weeks)			
	2	4	8	12
F_v/Fm				
Mg1	0.774 ± 0.001***	0.755 ± 0.002***	0.725 ± 0.003***	0.752 ± 0.003***
Mg10	0.790 ± 0.004	0.796 ± 0.002*	0.821 ± 0.002	0.790 ± 0.008
Mg20	0.784 ± 0.001*	0.785 ± 0.003	0.830 ± 0.002	0.779 ± 0.017
Mg50	0.788 ± 0.001	0.786 ± 0.002	0.827 ± 0.002	0.774 ± 0.003
Mg80	0.799 ± 0.002*	0.777 ± 0.002*	0.820 ± 0.002	0.778 ± 0.005
Mg200	0.794 ± 0.001	0.789 ± 0.002	0.809 ± 0.002***	0.766 ± 0.009
F_0				
Mg1	579 ± 17	583 ± 8***	438 ± 8***	558 ± 25
Mg10	539 ± 12	513 ± 8	375 ± 5**	597 ± 24
Mg20	556 ± 12	518 ± 7	355 ± 8	571 ± 14
Mg50	544 ± 13	514 ± 5	343 ± 5	514 ± 44
Mg80	541 ± 8	520 ± 5	358 ± 7	555 ± 8
Mg200	546 ± 6	518 ± 8	341 ± 10	599 ± 62
F_m				
Mg1	2620 ± 60	2498 ± 52	1622 ± 43***	2415 ± 107
Mg10	2521 ± 95	2539 ± 51	2104 ± 31***	2852 ± 123
Mg20	2609 ± 55	2416 ± 46	2090 ± 42	2897 ± 115
Mg50	2584 ± 63	2428 ± 52	1975 ± 28	2548 ± 267
Mg80	2616 ± 28	2348 ± 41	1868 ± 35	2484 ± 70
Mg200	2610 ± 62	2494 ± 62	1710 ± 67**	2426 ± 91

high irradiance, such a reduction in enzymatic activity could result in over-energisation and over-reduction of thylakoids and thus cause oxidative stress. According to Fischer (1997), accumulation of starch and soluble saccharides leads to disruption of protein turnover (especially protein D1 of the PSII reaction centre), causing an overall reduction in net CO_2 assimilation (Fischer 1997). Addition of extracellular Mg eliminated the enhancement effect of illumination on *in vitro* chloroplast phosphorylation and CO_2 fixation (Lin and Nobel 1971). All these studies support the idea of non-stomatal limitation of photosynthesis being a dominating component.

Applicability of IRGA measurements for screening

Based on our results, it is not likely that leaf gas-exchange parameters may be used as a screening tool for early diagnosis of Mg deficiency in plants or to select tolerant varieties. Measured at the age of 4 weeks, Mg-deficient plants (Mg1 and Mg10) performed even better than control plants (Mg50) when judged by A or g_s values (Figs 1*A*, 2*A*). A month later, light response curves of photosynthesis showed relatively strong correlation with leaf Mg status (Table 2; Fig. 1*B*). However, even at this age it was difficult to distinguish between moderately deficient Mg10 and optimal Mg50 variants (Fig. 1*B*). In addition, there are at least two confounding factors that make application of the IRGA (Infrared Gas Analyser) technique impractical for screening plants for Mg deficiency. First, on average, between 20 and 30 min are required to obtain a light curve of photosynthesis for one replicate. This is far too long and unproductive for the large numbers (hundred samples) screened. Second, during this process, leaf temperature in the chamber will inevitably rise (between 4 and 6°C, according to our data). That may be a reason for the observed decline in g_s values at highest PPFD of some treatments (Fig. 2*A*). Rigorous temperature control is impractical. The only way to overcome both of these problems is to measure leaf gas exchange parameters only at saturating PPFD. Even in this situation, some controversy remains (see above section), and IRGA measurements do not allow differences between moderately deficient (Mg10) and control (Mg50) plants to be distinguished (Fig. 2*B*).

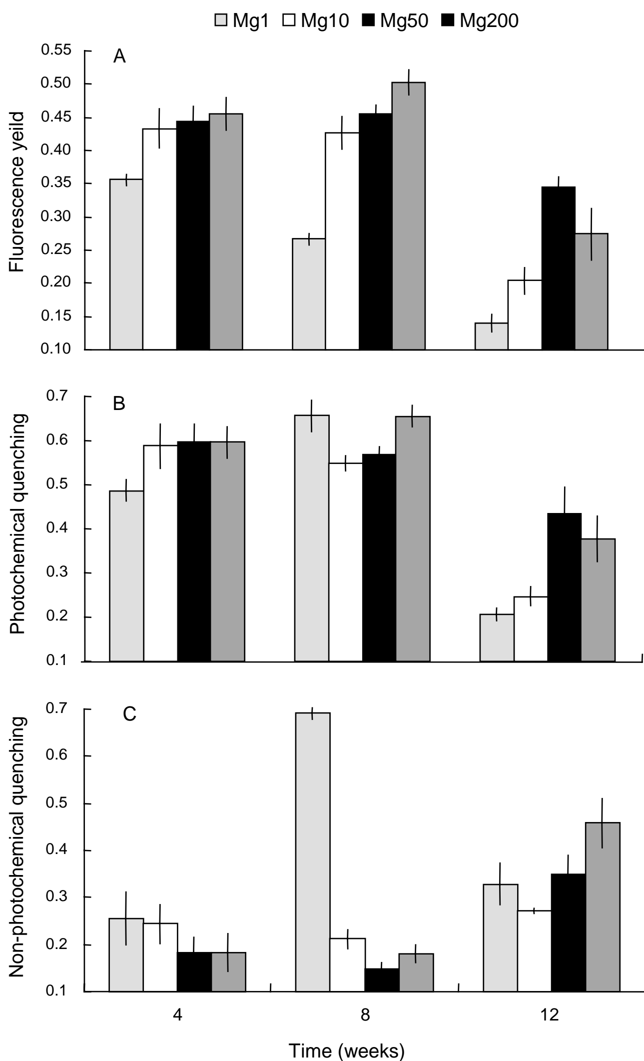

Fig. 4. Chlorophyll fluorescence parameters measured from the leaves of light-adapted broad bean plants after 4, 8, and 12 weeks of plant growth at various concentrations of magnesium. Data are average ± SE (n = 8–12). Y, quantum yield of PSII; qP, photochemical quenching; qN, non-photochemical quenching.

Chlorophyll fluorescence in plant screening for Mg deficiency

Despite chlorophyll fluorescence becoming a popular tool in plant screening for a wide range of environmental constraints (Haldimann *et al*. 1996; Maxwell and Johnson 2000; Shabala 2002), only a handful of studies applied this technique to address the issue of Mg deficiency in plants. Reports are rather controversial. Lasa *et al*. (2000) showed that 2 weeks of treatment with low (0.1 mM) Mg had no effect on either F_v/F_m or quenching characteristics in sunflower plants. In *Pinus radiata*, reduced rates of light-saturated photosynthesis and stomatal conductance were not accompanied by reduced quantum efficiency of PSII, although some reduction in F_v/F_m was observed for Mg-deficient needles (Sun *et al*. 2001). However, Laing *et al*. (2000) reported that, also in pine, photochemical yield (both F_v/F_m and Y) was significantly reduced by low Mg nutrition, while F_0 was unaffected. Laing *et al*. (2000) also found a strong and significant effect of Mg on electron transport rate (ETR) and near doubling of NPQ (non-photochemical quenching) at low Mg compared with control at low PPFD, while qP was not affected.

Our results indicated that as early as 2 weeks after the start of experiments, severely Mg-deficient plants (Mg1) had significantly lower maximum photochemical efficiency of PSII (F_v/F_m; Table 3) than control (Mg50) plants. At any

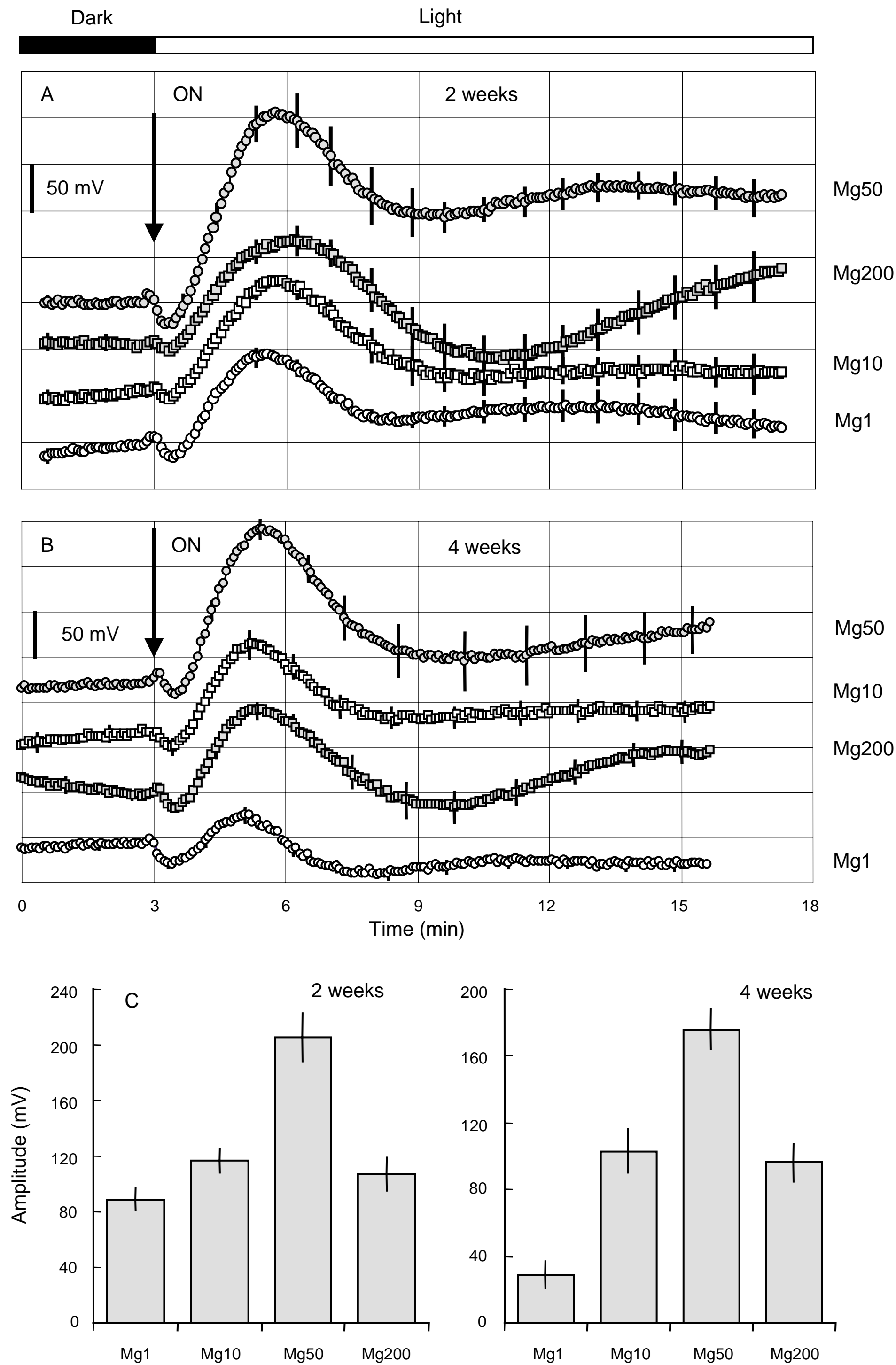

Fig. 5. Light-induced responses of leaf surface electrical potentials from the 5th leaf of broad bean plants, grown at different level of magnesium in the growth solution. White light (900 W m^{-2}) was given at the moment indicated by an arrow. (*A*) and (*B*) show transient response kinetics measured from 2- and 4-week-old plants, respectively. (*C*) The magnitude of leaf bioelectric response to light (measured as a difference between steady-state value and a peak value at maximum hyperpolarisation) as a function of plant age and Mg availability. Data are average ± SE (n = 8–12).

age, the F_v/F_m value remain a sensitive indicator of Mg deficiency in broad beans, although only for severe deficiency cases. For the cases of moderate deficiency (Mg10), F_v/F_m was not always as sensitive (Table 3). Equally, measured from light-adapted samples, photochemical efficiency *Y* was found to be a sensitive indicator of plant Mg status (Fig. 4). It may be suggested, therefore, that at high PPFD, a high proportion of reaction centers of Mg-deficient leaves were closed (photochemically inactivated through photoinhibition). However, although some trend became evident as early as 4 weeks, a significant (P=0.05) difference between Mg10 and Mg50 treatments was not observed until the end of the experiment, at the age of 12 weeks. This is consistent with findings by Laing *et al*. (2000) who reported no changes in *Y* parameters measured from pine needles at Mg concentration in the needle above 0.2 mg g^{-1} DW. This is not surprising as, according to some data, in Mg-deficient leaves, photosynthesis is affected much later than the development of visual deficiency symptoms (Peaslee and Moss 1966; Fischer and Bremer 1993).

Leaf electric measurements as a screening tool

The overall assessment of plant 'health' can be achieved by measuring the plasma membrane potential (MP) in leaf or root cells. In most plants, grown in optimal conditions, cell membrane potential is within –120 to –160 mV range. When plants are stressed, a significant depolarization occurs, and membrane potential values become much less negative (around –40 to –60 mV). Examples include salinity (Shabala *et al*. 2003), acid stress (Babourina *et al*. 2001), herbicide application (Di Tomaso *et al*. 1991) and drought stress (Hedrich *et al*. 2001).

To measure cell membrane potential, a fine microelectrode (tip size < 0.5 µm) is impaled into the cell under a microscope (Shabala and Newman 1999). Usually, only the outer cell layers can be measured, such as leaf or root

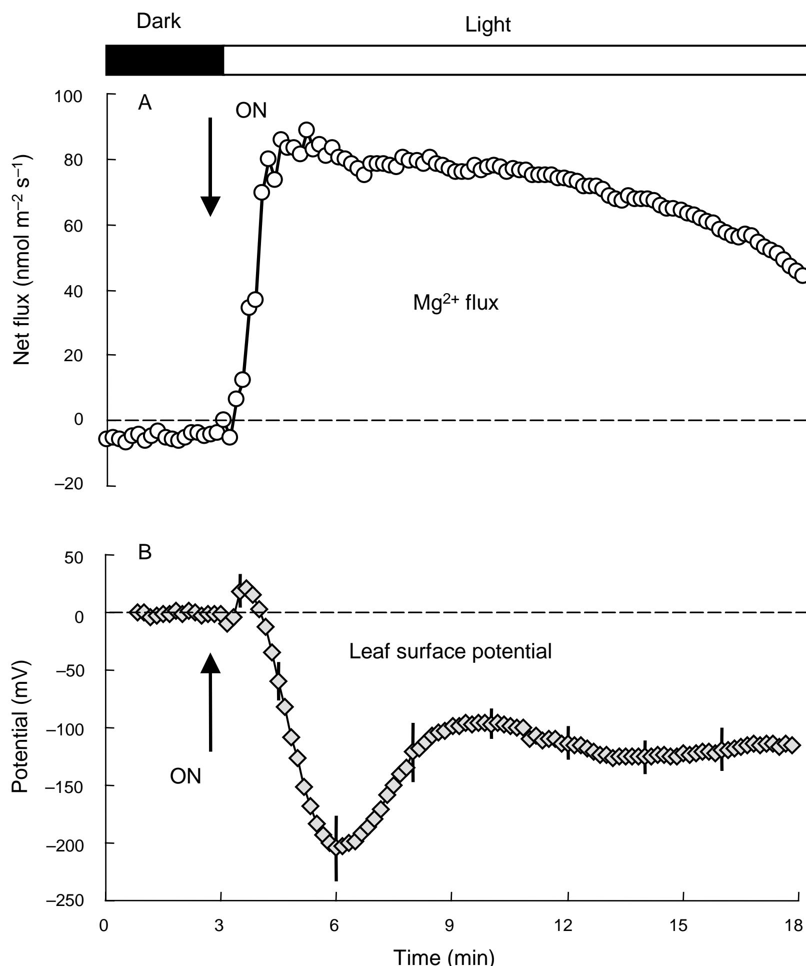

Fig. 6. (*A*) Light-induced Mg^{2+} fluxes measured from isolated mesophyll segments, taken from the 5th broad bean leaf of 1-month-old plants, grown at optimal (Mg50) magnesium supply. (*B*) Changes in the leaf surface potential for the same leaf. Data are average ± SE (n = 6).

epidermis. As the microelectrode tip may be rapidly clogged by the dense cytosol, the electrode needs frequent replacement. Therefore, it is very unlikely that this technique may be considered for plant screening for Mg deficiency.

A viable alternative to microelectrode MP measurements is to measure an integrated collective response of hundred of cells on the leaf surface by using the leaf surface potential measuring technique (Shabala 1997). Such an approach has already been used successfully in our laboratory for rapid diagnosis of plant responses to salinity (Shabala *et al*. 1998). It was shown that a promising method was to grow plants under different conditions and than challenge them with some [non-damaging] environmental factor, such as light. It is well known that chlorophyll-containing tissue reacts to light variations by changes in the surface electric potential. Being the composite response of hundreds of cells with different electrical properties (Girildini 1988; Shabala 1997), these responses incorporate information about the underlying bioelectric processes in the thylakoid (Peters and Berkowitz 1991; Bulychev and Vredenberg 1995) and plasma (Vanselow *et al*. 1989; Elzenga *et al*. 1995) membranes. Therefore, it was expected that leaf Mg status might

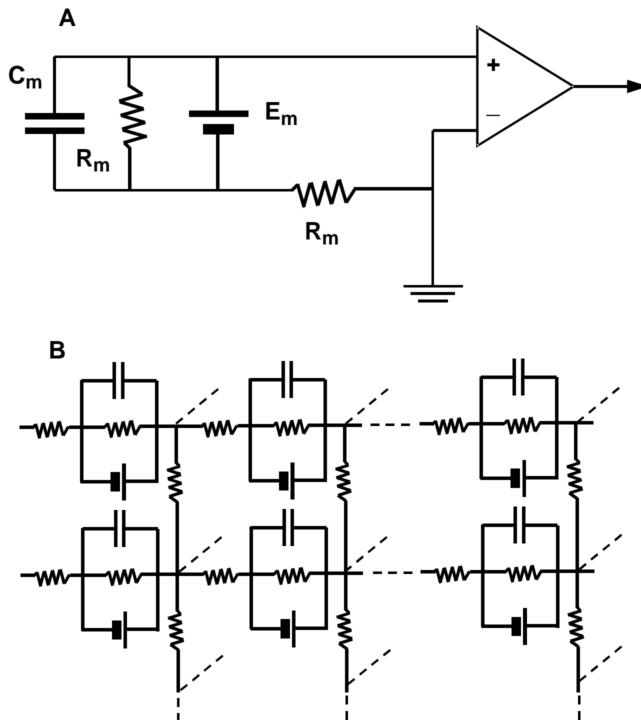

Fig. 7. (*A*) Equivalent electric circuit for a single cell. C_m, plasma membrane capacitance; R_m, plasma membrane resistance; R_a, resistance of the apoplast; E_m, electromotive force. During the dark-light transition, significant changes in E_m occur. (*B*) A three-dimensional analogue for the whole plant measured by extracellular electrode technique employed in this study. Bioelectric changes on the leaf surface are a collective response of hundreds of cells differing in their ability to respond to light.

be reflected in variations in light-induced kinetics of leaf surface potentials.

In the present study, we showed that a significant difference in the magnitude of leaf electrical responses to light, measured by means of the surface potential technique, can be found between optimal (Mg50) and deficient leaves as early as 2 weeks after the beginning of the experiment (Fig. 5). Not only severe (Mg1), but also moderately deficient (Mg10) leaves showed a significantly lower magnitude of response (Fig. 5*C*). At the age of 4 weeks, it became possible to order all treatments according to their predicted bean yield (see Fig. 1 in Hariadi and Shabala 2004). Therefore, it appears that leaf electric measurements may be used as an effective screening tool for Mg deficiency, at least in broad bean plants. Applicability of this technique to other species need more rigorous testing, as Mg deficiency symptoms show a high degree of species-dependence, and physiological responses to Mg may vary significantly between annual and perennial species (Marschner 1995).

Mg^+ fluxes across the plasma membrane as a potential contributor to leaf surface potential changes

As commented in our previous publication, the simplicity of the technique for making extracellular bioelectrical measurements is balanced by the complexity of the interpretation of results obtained by the technique (Shabala *et al*. 1998). Bioelectric changes on the leaf surface are contributed to by hundreds of cells differing in their electrical properties and in their ability to respond to light. At the cellular level, each cell may be considered as a source of electromotive force (E_m), having some capacitance (C_m) and resistance, both symplastic (R_m) and apoplastic (R_a, Fig. 7*A*). At the tissue level, however, hundreds and thousands of cells are connected together, forming a complex three-dimensional structure (Fig. 7*B*). When the surface electrode is attached to the leaf, a collective electric response of thousands of electrically coupled cells is measured (Girildini 1988; Shabala 1997).

During the dark-light transition, significant changes in leaf membrane potential (and, therefore, E_m) occur (Spalding *et al*. 1992; Elzenga *et al*. 1995; Shabala and Newman 1999). Each of the ions, moving across the plasma membrane, may contribute to this process. Fluxes of several ions, including Ca^{2+}, K^+, H^+ and Cl^-, were shown to be modulated by light-dark fluctuations (Spalding *et al*. 1992; Shabala and Newman 1999). Fluxes of each of these may be affected by Mg availability for plants. In this study, we showed that onset of illumination caused a significant and prolonged uptake of Mg^{2+} across the plasma membrane into mesophyll cells (Fig. 6*A*). The similarity between Mg^{2+} flux (Fig. 6*A*) and leaf surface potential (Fig. 6*B*) kinetics suggests that magnesium concentration in the apoplast and, therefore, light-induced changes in Mg^{2+} uptake into the cytosol, may be one of the reasons for a pronounced

difference in the magnitude of leaf surface potential responses to light (Fig. 5).

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